Matrix metalloproteinase degradation of extracellular matrix: biological consequences

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Targeted mutagenesis has allowed investigators to perform controlled experiments in mammals and determine the contribution of individual proteins to physiologic and pathologic processes. Recent lessons learned from matrix metalloproteinase gene targeted mice and other *in vivo* observations have given new life to old concepts regarding the role of proteolytic fragments of extracellular matrix proteins in regulating a variety of critical processes in cell biology

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Abbreviations

ADAM	a disintegrin and metalloproteinase domain
APC	adenomatous polyposis coli
ApoE	apolipoprotein E
ECM	extracellular matrix
LLC	Lewis lung cell carcinoma
MMP	matrix metalloproteinase
PGK	phosphoglycerol kinase
TIMP	tissue inhibitor of metalloproteinases
TNF	tumor necrosis factor α
t-PA	tissue-type plasminogen activators
u-PA	urokinase-type plasminogen activators

Introduction

Matrix metalloproteinases (MMPs) comprise a family of extracellular matrix degrading enzymes (Table 1) that are believed to play pivotal roles in embryonic development and growth as well as in tissue remodeling and repair. Excessive or inappropriate expression of MMPs may contribute to the pathogenesis of many tissue-destructive processes, including highly prevalent diseases such as arthritis, multiple sclerosis, and tooth decay, as well as the leading causes of death in developed countries: cardiovascular disease (atherosclerosis plaque rupture and aneurysm formation), tumor progression, and chronic obstructive pulmonary disease'. A statement such as this is usually found at the beginning of MMP-related papers (and of course grant applications — simply insert disease of interest) written by those of us with a 'metallocentric' view of the world [1•]. But judgment day is approaching. With the advent of targeted mutagenesis, one can perform controlled experiments in mammals to test these hypotheses. Moreover because these important diseases can potentially be attributable to the action of MMPs, effective synthetic MMP inhibitors are being developed and are rapidly approaching clinical trials [1•,2].

Targeted mutagenesis of MMPs

Over the past couple of years, many of the MMPs have undergone gene-targeting experiments (Table 2). The power of gene targeting was best summarized by Piagen, who stated, "one invariable lesson of biological research has been the difficulty, virtual impossibility, of reliably predicting the properties of intact organisms from the properties of their constituent tissues, cells and molecules. Thus, hypotheses need to be confirmed in intact, complex biological organisms; not prokaryotes or lower eukaryotes, but mammals [3]." One must, of course, recognize the limitations of these experiments. First, loss of a protein from the blastocyst stage onward might alter complex biological processes, leading to what is commonly referred to as compensation. Second, because of gene redundancy, mutation of a gene may not unmask the true biological function of the protein it encodes. Third, mice are not humans; hence, direct translation of results to human biology requires knowledge of biological similarities and differences between these species [4].

When interpreting data from gene-targeting experiments one must also be aware of potential strain differences and the possibility of 'neighborhood knock-out' effects. This term refers to inhibited expression of genes physically linked to the target gene, and is probably related to the retention of the phosphoglycerol kinase (PGK) promoter used to drive selectable markers [5]. This is a pertinent concern because several MMP genes (collagenases, stromelysins, matrilysin, and macrophage elastase) are closely linked on human chromosome 11q22 and mouse chromosome 9. With these caveats in mind, what have MMP-mutant mice told us about the biological consequences of MMP-mediated extracellular matrix (ECM) degradation?

Physiological processes

MMPs, usually undetectable in cells under normal circumstances, are prominently expressed during a variety of biological processes, such as reproduction. On the maternal side, MMP expression is associated with menstruation, ovulation, uterine implantation, parturition, and postpartum uterine and mammary gland involution [6]. From the offspring's perspective, MMPs are believed to be required for trophoblast implantation, embryonic growth, and tissue morphogenesis. Yet, none of the individual MMP-mutant mice generated to date have had an embryonic lethal phenotype. Mice deficient in gelatinase B (MMP-9$^{-/-}$) demonstrate morphologic abnormalities at the site of implantation, but these defects are not lethal. All MMP-deficient mice to date are capable of delivering and nurturing healthy pups.

Table 1

Matrix metalloproteinase substrates*.

MMP	Interstitial collagens	Basement membrane	Elastin	Non-matrix proteins
Collagenases				
MMP-1	III > I >(+/– II); VII, X	+/– FN, LN, EN, PG	–	L-selectin is a substrate for collagenases
MMP-8	I > III > II, (? VII, X)	+/– FN, LN, EN, PG	–	
MMP-13	II > I, III, GL (? VII, X) and telopeptidase	+/– FN, LN, EN, PG	–	
Stromelysins				
MMP-3	–	FN, LN, EN, PG, +/– PS col IV	+/–	EGF-like growth factor and plasminogen are substrates for stromelysins
MMP-10†	–	FN, LN, EN, PG, +/– PS col IV	+/–	
Stromelysin-like				
MMP-7	–	FN, LN, EN, PG	+	Stromelysin-like enzymes are most potent at converting plasminogen to angiostatin and degrading α_1 AT
MMP-12	–	FN, LN, EN, PG, PS col IV	++	
Gelatinases				
MMP-2	GL, I, VII, X, XI	col IV/V, FN, LN, EN, PG, PS	++	
MMP-9	GL	col IV/V, FN, LN, EN, PG, PS	++	
Furin-recognition sites				
MMP-11‡	–	–	–	
Membrane type				
MMP-14§	+/– I > III, II	FN, LN, EN, PG		
MMP-15		FN, LN, EN, PG		
MMP-16	?			
MMP-17	?			
Newly described				
Enamolsin	GL (amelogenin)	?	?	?

*Note this list is inherently incomplete, representing only selected substrates tested to date. These substrates guide potential biological functions, but the actual *in vivo* substrates are unknown. †MMP-10 has the same substrate specificities as MMP-3 but is less potent. ‡Human enzyme not catalytically active to known ECM components. §Soluble recombinant protein was tested. α_1 AT, α_1 antitrypsin, EN, entactin; FN, fibronectin; GL, gelatin, colIV/V, types IV and V collagen), LN, laminin; PG, proteoglycan, PS col IV, pepsinized type IV collagen.

Post-natal development

The major defect in MMP-9$^{-/-}$-deficient mice is delayed long bone growth and development [7*]. Long bones develop from mesenchymal condensations where cartilage cells differentiate and deposit a cartilage matrix. Blood vessels invade and degrade the cartilage matrix, and cartilage cells undergo apoptosis followed by proliferation of osteoblasts and endochondral ossification, which converts the tissue into mature bone. In MMP-9$^{-/-}$ mice, there is delayed vascular invasion of skeletal growth plates, resulting in an excessively wide zone of hypertrophic cartilage and delayed ossification. MMP-9 is required to initiate primary angiogenesis in the cartilage growth plate, probably through generation of an angiogenic signal (or perhaps degradation of an angiogenesis inhibitor). Interestingly, the mechanism of this phenotype may not involve degradation of a structural or adhesive matrix protein.

While this phenotype is marked during growth, if one were merely to study adults only a 10% shortening in the long bones would be appreciated. This is not meant to diminish the importance of this finding but, rather, it emphasizes that until careful analyses of all MMP$^{-/-}$ mice are performed conclusions regarding the role of individual MMPs in growth and development are premature. The overall minimal phenotypes observed to date may be due to redundancy, safeguarding the host from untoward consequences of individual MMP mutations. Generation of doubly and multiply MMP deficient mice may be required to unmask full MMP function. Alternatively, MMPs may not be needed for grossly normal development and growth in the mouse.

MMP-9 was demonstrated in the mesenchyme of embryonic kidneys, and branching morphogenesis of ureteric buds was specifically blocked in metanephric organ culture by antisera to MMP-9 but not by IgG antibodies to MMP-2 [8]. No abnormalities were found upon analysis of neonatal and adult MMP-9$^{-/-}$ mice by light microscopy or immunofluorescence for basement membrane proteins, however, and renal function in adult mice was normal [9]. It is not clear whether the discrepancies between these studies result from differences in study design, *in vivo* versus *in vitro* studies, or whether antibody experiments overestimate the consequences of gene inactivation while gene knockout experiments underestimate them.

Table 2

Phenotypes of MMP-deficient and related null mutant mice.

Mice	Result	Reference
MMP- deficient		
Gelatinase A (MMP-2)	Unaltered secretion of β-amyloid precursor protein	[49]
	Reduced angiogenesis and tumor progression	[21]
Stromelysin-1 (MMP-3)	No effect on collagen-induced arthritis	[50]
Matrilysin (MMP-7)	Decreased intestinal tumorigenesis	[22*]
Gelatinase B (MMP-9)	Impaired primary angiogenesis in bone growth plates	[7*]
	Resistant to bullous pemphigoid	[41]
Stromelysin 3 (MMP-11)	Decreased chemical-induced mutagenesis	[23*]
Macrophage elastase (MMP-12)	Impaired macrophage proteolysis	[14]
	Impaired macrophage recruitment and protection from cigarette-smoke-induced emphysema	[37*]
Related null mutants		
TIMP-1	Loss of TIMP-1 in transformed cell lines can either potentiate or suppress frequency of tumor invasion	[26]
Point mutation (cleavage site in type I collagen)	Marked dermal fibrosis	[51]
	Impaired post partum uterine involution	
	MMP-13 N-telopeptide cleavage accounts for bone resorption during embryonic and early adult life	
u-PA	Unable to activate pro-MMPs	[13*]
	On ApoE-/- background, protected from atherosclerotic macrophage infiltration and microaneurysm formation	

Pathological processes

Cardiovascular disease

Atherosclerosis is a chronic inflammatory process whereby plaques are formed in the intimal layer of the vessel wall as a result of accumulation of ECM, smooth muscle cells, and lipid-laden macrophages. In humans, coronary artery plaques may become unstable and rupture, triggering intravascular thrombosis leading to myocardial infarction. The atherosclerotic vessel wall may also dilate as a result of destruction of the medial elastic lamina, leading to aneurysm formation and rupture of the weakened vessel wall. Recently, plasminogen activators and several MMPs have been detected in association with human atherosclerotic arteries [10] and abdominal aortic aneurysms [11]

Mice with a targeted disruption of the apolipoprotein E (ApoE) gene have a delayed clearance of lipoproteins from the blood. When mice are fed a Western diet serum cholesterol levels reach 1400–2000 mg/dl, and fatty streaks progressing to fibrous plaques develop at branch points of major vessels. The formation of these lesions is associated with macrophage recruitment causing disruption of the medial elastic lamina and microaneurysm formation. Complex lesions with plaque rupture and hemorrhage have yet to be observed for any model of atherosclerosis in the mouse (for a review see [12])

To investigate the role of plasminogen activators (tissue-type plasminogen activators [t-PA] and urokinase-type plasminogen activators [u-PA]), Carmeliet and colleagues [13*] crossed $ApoE^{-/-}$ mice with $u\text{-}PA^{-/-}$ or $t\text{-}PA^{-/-}$ mice. $ApoE^{-/-} \times u\text{-}PA^{-/-}$ mice, but not $ApoE^{-/-}$ or $ApoE^{-/-} \times t\text{-}PA^{-/-}$ mice, were protected from macrophage-mediated destruction of medial elastic lamina and microaneurysm formation. Macrophages lined up along elastic lamina but they did not penetrate or disrupt these matrix structures. Because the ability of macrophages to degrade and migrate through elastin is more likely due to macrophage elastase (MMP-12) [14] than to non-elastolytic plasmin, these findings suggest that plasmin may activate MMP pro-enzymes, an old hypothesis not previously demonstrated *in vivo*. Indeed, in the absence of u-PA, macrophages were unable to convert macrophage pro-MMPs (MMP-3, MMP-9, MMP-12, and MMP-13) into their active forms in a reconstituted system [13*].

In contrast, in the human stromelysin-1 (MMP-3) promoter, a genetic polymorphism which causes diminished stromelysin-1 expression is associated with enhanced progression of atherosclerosis [15] Together these and other studies suggest that MMPs initially maintain patency of the atherosclerotic vascular lumen at the risk of subsequent plaque rupture

Cancer

MMPs are believed to promote tumor progression by initiating carcinogenesis, enhancing tumor angiogenesis, disrupting local tissue architecture to allow tumor growth, and breaking down basement membrane barriers for metastatic spread [16,17]. While some MMPs, such as matrilysin (MMP-7) collagenase-3 (MMP-13), and often gelatinase A (MMP-2), are expressed by tumors cells themselves, MMPs are predominantly produced by surrounding host stromal and inflammatory cells in response to factors released by tumors [17,18*] MMPs may then bind to tumor cells and angiogenic endothelial cells, advancing tumor progression. For example, MMP-2 binds through its carboxy-terminal domain to $\alpha v\beta 3$ integrin on melanoma cells and angiogenic blood vessels, enhancing tumor growth [19]. Autolytic processing of MMP-2 with release of the carboxy-terminal domain competes with cell surface binding of the enzyme, inhibiting angiogenesis and tumor growth [20*]. Consistent with these results, $MMP\text{-}2^{-/-}$ host mice exhibit impaired primary tumor growth and

decreased experimental metastases of B16-BL6 melanoma and Lewis lung cell carcinoma (LLC) cells [21].

Matrilysin (MMP-7) is expressed by tumor cells derived from gastrointestinal epithelium, including those that arise spontaneously in mice with the adenomatous polyposis coli (APC) multiple intestinal neoplasia mutation APC^{min}. MMP-7-/- × APC^{min} mice had delayed tumor development [22•]. Similarly, stromelysin-3 deficient (MMP-11-/-) mice demonstrated impaired tumor formation in response to chemical mutagenesis [23•]. These studies confirm a role for MMPs in carcinogenesis. Potentially, ectopic expression of these proteinases (in combination with another mutation) may release cells from ECM-mediated cell cycle arrest. Alternatively, proteinases may release growth or angiogenesis factors promoting tumorigenesis.

While overexpression of tissue inhibitors of metalloproteinases (TIMPs), either in transgenic mice or by gene transfer, can decrease tumor progression in animal models [24,25], it has been difficult to demonstrate that lack of TIMP-1 in tumor or host consistently enhances tumor growth [26]. Perhaps expression of TIMPs 2–4 compensate for loss TIMP-1 expression in these models.

While MMPs commonly facilitate tumor progression, proteolytic cleavage products of MMPs may inhibit angiogenesis, limiting tumor progression. This was first apparent with the isolation of angiostatin from the urine of mice with LLC cells [27]. Angiostatin, a plasminogen cleavage product containing kringle domains 1–4, inhibits endothelial cell proliferation and is believed to be responsible for maintaining LLC lung metastases in a dormant state [27].

Generation of angiostatin in primary LLC tumors correlated with the presence of macrophages and macrophage elastase (MMP-12) [28]. The importance of MMP-12 in limiting lung metastasis growth in the LLC model has been confirmed by use of mice rendered deficient in macrophage elastase (MMP-12-/-) by gene targeting (JL Gisolano and SD Shapiro, unpublished data). Preliminary studies suggest, however, that local expression of MMP-12 in macrophages surrounding the secondary lung metastases limits growth, in part through generation of angiostatin. This effect may also be related to MMP-2 processing by MMP-12 or other mechanisms.

Several other MMPs are also capable of generating angiostatin and other antiangiogenic fragments of plasminogen [29,30]. The kringle 5 domain by itself appears to have the greatest capacity to inhibit endothelial cell proliferation [31]. Serine proteinases, including plasmin, in association with an extracellular reductase that reduces disulfide bonds in plasmin, also trigger generation of angiostatin [32•,33]. In addition to angiostatin, other proteolytic fragments most prominently endostatin (a 20 kDa carboxy-terminal fragment of type XVIII collagen), effectively inhibit tumor angiogenesis [34•]. In fact, treatment of several tumors in mice with endostatin resulted in prolonged tumor dormancy [35•].

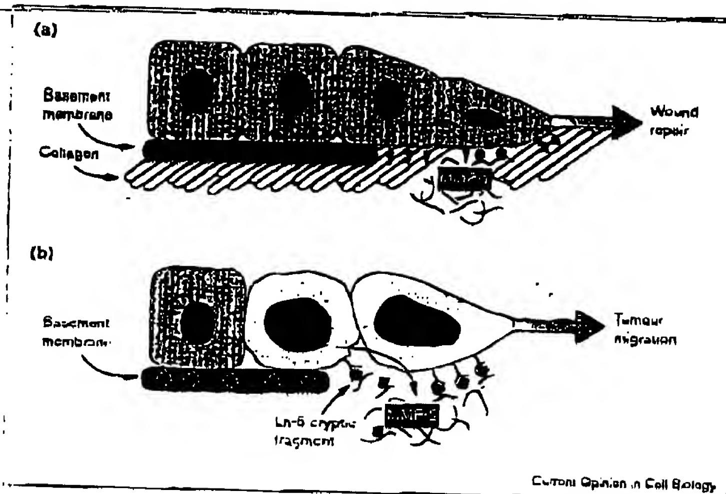
Thus, proteinases may benefit the host or the tumor depending upon spatial expression, proteolytic capacity, and binding affinity for matrix and tumor cells. Nevertheless, hydroxamates, which are MMP zinc-chelating agents, are effective in inhibiting growth of several primary tumors and metastases in animal models [1•] including LLC [36]. The ability of these compounds to inhibit growth of neoplasms suggests that tumors use MMPs more effectively than the host; when all the MMPs are inhibited the host has the advantage. MMP inhibition combined with anti-angiogenic agents, such as angiostatin, endostatin, or $\alpha v\beta 3$ integrin, might prove optimal in clinical treatment of particular tumors.

Pulmonary emphysema

A major component of chronic obstructive pulmonary disease is destruction and enlargement of peripheral airspaces of the lung. Chronic exposure to cigarette smoke exposure leads to inflammatory cell recruitment and activation with release of elastases, in excess of inhibitors. ECM degradation coupled with abnormal repair results in lung destruction characteristic of emphysema. The serine proteinase neutrophil elastase is responsible for emphysema in patients with a genetic deficiency of its inhibitor α_1-antitrypsin, a relatively uncommon form of the disease; however, the contribution of neutrophil elastase to the more common emphysema associated with cigarette smoking is controversial. It is possible that other neutrophil proteinases or enzymes from the more abundant macrophages contribute to lung damage associated with prolonged cigarette smoking.

Long-term exposure of wild-type (MMP-12+/+) mice to cigarette smoke led to inflammatory cell recruitment followed by alveolar space enlargement similar to the pathologic defect in humans. Mice deficient in macrophage elastase (MMP-12-/-), however were protected from development of emphysema despite heavy long-term exposure to smoke. Surprisingly, MMP-12-/- mice also failed to recruit monocytes into their lungs in response to cigarette smoke [37•]. Because MMP-12 and most other MMPs are only expressed upon differentiation of monocytes to macrophages, it appeared unlikely that monocytes require MMP-12 for transvascular migration. More likely, cigarette smoke induces constitutive macrophages, which are present in lungs of MMP-12-/- mice, to produce MMP-12 that in turn cleaves elastin, thereby generating fragments chemotactic for monocytes (JP Paige and SD Shapiro, unpublished data). This positive feedback loop would perpetuate macrophage accumulation and lung destruction. The concept that proteolytically generated elastin fragments mediate monocyte chemotaxis was first shown more than a decade ago [38–40].

Figure 1



ECM-mediated cell migration. **(a)** Keratinocyte migration during normal wound healing. Exposure of keratinocytes to interstitial collagen in the provisional matrix leads to high affinity binding (arrows) of α2β1 integrin to collagen. This leads to expression of MMP-1, which degrades collagen and frees the cell to migrate, perhaps using other receptors (circles). α2β1 binding to collagen at the migrating front 'pulls' the cell forwards leading to re-epithelialization (adapted from [1•]). **(b)** Epithelium-derived tumor cell migration. Tumor cells (lightly shaded) express MMP-2 (or induce stromal cells to produce it), which degrades the basement membrane (wiggly lines), exposing cryptic fragments of laminin and collagen which are only exposed upon degradation (diamonds). These bind to cell receptors leading to migration perhaps related to integrin-mediated signaling leading to cytoskeletal changes causing cell movement. Ln5, laminin 5.

Bullous pemphigoid

The autoimmune subepidermal blistering disease known as bullous pemphigoid is characterized by deposition of autoantibodies at the basement membrane zone. In an experimental model of this disease in mice, the blistering is mediated by antibodies directed against the hemidesmosomal protein BP180 (collagen XVII), and depends on complement activation and neutrophil infiltration. In contrast to wild-type littermates, MMP-9-/- mice were resistant to the blistering effects in this model despite deposition of autoantibodies and neutrophil recruitment equivalent to that seen in wild-type mice [41]. Whether MMP-9 directly causes blistering or augments neutrophil elastase activity by degrading α_1-antitrypsin is currently unknown.

Bioactivity of ECM fragments

Proteolytically generated ECM (and non-ECM) fragments have long been thought to regulate a diverse array of processes in cell biology. The importance of this mode of regulation has been a recurrent theme in the recent *in vivo* studies presented here: plasmin mediates MMP activation; plasminogen fragments (angiostatin and other kringle domains) and collagen XVIII fragments (endostatin) inhibit neovascularization, while MMP-9 induces angiogenesis; elastin fragments may regulate monocyte recruitment in chronic inflammation.

Overexpression of stromelysin-1 (MMP-3) in mammary glands of transgenic mice not only confirmed the expected role of MMPs in mammary gland branching morphogenesis, but unexpectedly demonstrated an additional role in regulating post-partum mammary gland involution [42]. A series of subsequent studies demonstrated that cell contact with correct tissue architecture is crucial for cell homeostasis, suppression of apoptosis, and maintenance of differentiated phenotype (see N Boudreau and MJ Bissell, pp 640–646).

It was also recently recognized that cleavage of the laminin-5 γ2 chain by gelatinase A (MMP-2) exposed a cryptic site within laminin, inducing migration of malignant breast epithelial cells [43•]. This study suggests that local proteinase concentration may determine cell behavior. Proteolysis is required to initiate and sustain migration, but excessive proteolysis may degrade matrix signals and receptors, thereby disrupting cell matrix interactions and inhibiting migration [1•]. With respect to epithelial cell migration in normal wound healing, Parks and co-workers [44•] hypothesized that interaction of keratinocyte α2β1 integrin with native type I collagen in a provisional wound matrix induces collagenase-1 (MMP-1) expression. By cleaving collagen, the initial high affinity contact is loosened, releasing the cell, which then migrates to 'grab' high affinity α2β1 integrin bonds with undigested collagen ahead in the open wound (Figure 1). Indeed, keratinocytes can migrate on native collagen but not on a collagenase-resistant collagen matrix [45•]. Similarly, in fibroblasts, binding of fibronectin fragments (but not intact fibronectin) to α5β1 integrin signals activator protein-1 (AP-1)-mediated induction of MMP-1 synthesis [46]. Thus, ECM provides an important mechanism for cells to communicate with their external environment. When cells are in contact with their appropriate, intact ECM they are quiescent (or at least perform their normal functions); however, cell contact with inappropriate, altered, or disrupted ECM sets in motion a variety of signal transduction pathways and gene transcription resulting in many cellular responses with the goal of tissue repair [47•].

In addition to MMPs, closely related metalloproteinases termed ADAMs (a disintegrin and metalloproteinase

domain) are well positioned on the cell surface to release or 'shed' a variety of important inflammatory cell mediators (see RA Black and JK White, pp 654–659). Earlier it was discovered that hydroxamic acid MMP inhibitors prevented release of latent tumor necrosis factor α (TNF) from monocyte surfaces, and subsequently the gene encoding the responsible proteinase, TACE (TNF convertase), or ADAM-18, was cloned. Metalloproteinases also mediate shedding of L-selectin, IL-6, Fas, TNF receptor, and a variety of other TNF receptor superfamily members. Additionally, significant stores of matrix bound transforming growth factor β may also be proteolytically released by plasmin and perhaps MMPs [48].

Conclusion and future prospects

Why are there so many MMPs? Overlapping but distinct substrate specificities and cell-specific expression suggest potentially unique functions, but clearly a variety of MMPs are expressed during development, perhaps to compensate for potential loss of an individual MMP. This diversity would explain why the phenotypes of MMP-mutant mice have been so mild with respect to development and other physiologic processes. Alternatively, MMPs may have a primary function in repair and defense, and in mature tissues, rather than a requisite function in morphogenesis. Further developmental analyses and mice with multiple MMP deficiencies will help address this issue. In contrast, aberrant or excessive expression of individual MMPs causes certain destructive diseases. Consequently, it has been easier to show that deletion of specific, abnormally expressed MMPs prevents disease onset. Greater understanding of similarities between humans and mice will guide rational medical therapy in the future. Gene-targeted mice will help investigators dissect molecular pathways and further define the role of proteolytic ECM (and non-ECM) cleavage products as regulators of gene transcription, angiogenesis, cell migration, inflammation, and cell cycle control, independent of translational research aspects.

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